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From F. T. Brooks

A DISEASE OF TOMATOES.

BY

F. T. BROOKS AND S. R. PRICE.

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By F. T. Brooks, M.A.
(Senior Demonstrator of Botany, Cambridge University),
and
S. R. Price, B.A.
(Frank Smart Student, Cambridge University).

[With 13 Text-Figs].

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In October, 1911, some diseased tomatoes grown out of doors in the neighbourhood of Bristol were sent to one of us for examination. These fruits bore large diseased areas somewhat depressed below the surface of the healthy parts. On the diseased portions of the fruits three fungi were found, a species of *Cladosporium*, a species of *Macrosporium*, and a fungus having pycnidial fructifications. These fungi were indiscriminately mixed for the most part, but on some of the diseased areas most recently formed only the fungus bearing pycnidia could be seen. It was thought, therefore, that the fungus with pycnidial fructifications might be the actual cause of the rot, the other fungi having appeared later as saprophytes.

In order to determine which organism was the cause of the disease the three fungi were isolated in the usual way by means of plate cultures. Tomato fruits approaching maturity were inoculated with these fungi in the following manner, one kind of fungus only being inserted in each of the fruits:—in opposite sides of each of three fruits two slits were made with a scalpel, and mycelium taken from a pure culture of each fungus was inserted in them, the fruits being placed on glass plates and covered with glass dishes. Three days later a rot had begun around each of the two slits in which the fungus with pycnidial fructifications had been placed, whereas the tomatoes inoculated with *Macrosporium* and *Cladosporium* respectively remained unaffected. After a fortnight the one tomato was completely rotted, whereas the other two were sound. Hence this preliminary trial made it tolerably clear that the fungus bearing pycnidia was the cause of the rot, the *Macrosporium* and *Cladosporium* being of no significance in this case, although it is well-known that species of both of these genera sometimes cause diseases of tomato fruits. Subsequent experiments with the three fungi confirmed the result of the preliminary trial, *viz.*, that the fungus having a pycnidial type of fructification was the cause of the disease.

At a later date one of a consignment of tomato fruits grown under glass in the neighbourhood of Cambridge was found to be affected with the same fungus, the pycnidia, spores, and characters when grown on artificial media being identical with those of the fungus isolated from tomatoes grown out of doors.

Identification of the Fungus on Tomato Fruits.

As yet few fungi with pycnidial fructifications have been described as causing diseases of tomato fruits. Plowright[1] in a paper on diseases of tomatoes mentions *Phoma destructiva* and *Sphæronema lycopersici* as causing rots of the fruit. The former fungus was associated with a species of *Cladosporium* and a species of *Macrosporium*, both of which Plowright considered to be stages in its life-history; but in the absence of culture and inoculation experiments it is impossible to say whether this was really the case or whether either of these forms was the actual cause of the rot. The characters of *Sphæronema lycopersici* as given by Plowright agree more closely with those of the fungus with which this paper deals and it is possible that they are identical, but Plowright's description of the fungus is too meagre to enable the identity to be established. Massee[2] mentions a pycnidial stage as occurring in the life-history of *Macrosporium solani*, Cooke, which is now considered to be identical with *Macrosporium tomato*, Cooke, the cause of the common black rot disease of tomato fruits, but the characters of the pycnidia do not agree with those of the fungus with which we are now concerned.

Massee has recently described the occurrence on tomato plants in England of the fungus *Diplodina citrullina*, Grossenbacher, or *Ascochyta citrullina*, C. O. Smith, as it is sometimes called, this fungus being the pycnidial stage of *Mycosphærella citrullina*, Grossenbacher. Massee[3] describes this fungus as attacking in an epidemic manner the stems of tomato plants grown under glass. One of us has also had the fungus under observation in Cambridgeshire where it has attacked plots of out-door tomatoes for two seasons in succession. In these cases the lower part of the stem was the region attacked, the cortex being partly destroyed. It occurred to us that the fungus on the tomato fruits might be identical with the *Ascochyta citrullina* mentioned above. The characters of the pycnidia and spores of the fungus on the fruit closely approached those of *Ascochyta citrullina* on the stems of

[1] Plowright, C. B. *The Gardeners' Chronicle*, 1881, p. 620.

[2] Massee, G. *Diseases of Cultivated Plants and Trees*, p. 503.

[3] Massee, G. *Kew Bulletin*, 1909.

tomato plants, and upon sending to Mr. Massee a specimen of the fungus on the fruit, he pronounced it to be "*Ascochyta citrullina*, C. O. Smith, the conidial form of *Mycosphærella citrullina*, Grossenbacher."

The young pycnidia on the tomato were pale brown in colour, and became darker with age, the spores were hyaline and occasionally uniseptate. The measurements both of the pycnidia and spores were about the same as those given by Massee in his description of *Ascochyta citrullina*. When mounted in water and observed under the microscope, mature pycnidia were seen to liberate their spores in a coil-like manner through the ostiole. The pycnidia obtained from the stems of tomato plants liberated their spores in the same manner.

As material of the fungus was available both from fruit and from stem it was thought desirable to grow the fungus from the two sources on artificial media and to perform inoculation experiments. The fungus obtained from the fruit was placed in the stems, and the fungus from the stem was placed in the fruits. In this manner it was hoped to establish more completely the identity of the fungus from the two sources.

Cultures of the Fungus Isolated from the Fruit.

On account of the method of liberation of the spores it was easy to obtain them in a fairly clean state and establish pure cultures of the fungus. The spores germinated readily both in water and in a dilute solution of sugar.

On tomato extract containing 10 per cent. of gelatine the fungus grew well, forming a dense white mycelium which began to sink into the medium after 8-10 days, at the same time causing liquefaction of the gelatine. The mycelium did not produce spores when grown on this medium.

On tomato-agar, *i.e.*, agar containing tomato extract, the mycelium grew less vigorously but pycnidia developed in considerable numbers. These fructifications were brownish in colour and similar in structure to those obtained on tomato fruits, but were more variable in size. The spores were similar to those produced by pycnidia that developed in the tissues of the plants. In old cultures the spores exuded from the pycnidia and formed blobs of a pinkish colour at their orifices, though the masses of spores sometimes spread over the surface of the pycnidia or the medium in the immediate vicinity. In these cultures there was often a tendency for the pycnidia to be distributed in concentric circles

similar to the manner in which other fungi develop their fructifications on various media, but this tendency was more marked in the case of certain cultures of the fungus originally derived from the stems of tomato plants.

On pure agar the fungus grew much less readily; the mycelium was sparse and only a few pycnidia developed. Fig. 1 represents

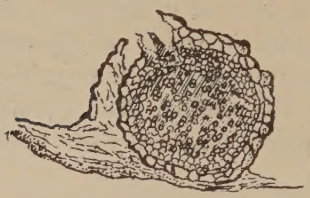

Fig. 1. Fungus isolated from fruit. Section of pycnidium on agar. ×125 diam.

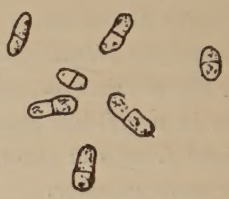

Fig. 2. Fungus isolated from fruit. Fresh spores from pycnidium on agar. ×770 diam.

a microtomed section of one of these pycnidia and Fig. 2 fresh spores from such a pycnidium.

Cultures of the Fungus Isolated from the Stem.

Cultures were obtained in the same manner as in the case of the fungus isolated from the fruit. The spores germinated readily both in water and in a dilute solution of sugar.

On tomato-gelatine a dense white mycelium developed which began to sink into the medium in about 8 days and caused liquefaction of the gelatine. No fructifications were formed in cultures on this medium.

On tomato-agar the mycelium grew less vigorously but numerous pycnidia developed. In appearance and structure these fructifications were identical with those which occurred in cultures of the fungus derived from the fruit. The spores, represented in Fig. 3, were similar and in old cultures were aggregated in the same manner at the orifices of the pycnidia. In these cultures also there was a tendency for the fructifications to develop in concentric circles and in one particular series of plates which were kept in a cool position in a North light during December this distribution was well marked, as is shown in Fig. 11, which is a photograph of one of these cultures. Although many other cultures were subsequently kept under approximately the same conditions, the "fairy ring" mode of distribution of the pycnidia was not again so well marked.

On pure agar the mycelial growth was very thin and only a

small number of pycnidia were formed. Fig. 4 represents a microtomed section of one of these pycnidia.

The above observations make it clear, therefore, that as regards behaviour on culture media the fungi derived from the fruit and stem respectively are identical.

Fig. 3. Fungus isolated from stem. Fresh spores from pycnidium on tomato agar. ×770 diam.

Fig. 4. Fungus isolated from stem. Section of pycnidium on agar. ×770 diam.

Inoculation Experiments with Fungus Derived from the Fruit.

Experiments showed that with green fruits infection would only result if the fungus, either in the form of mycelium from a pure culture on tomato-gelatine, or of spores, was inserted into wounded parts. In such inoculations a rot began in the immediate neighbourhood of the wound a few days after the fungus was

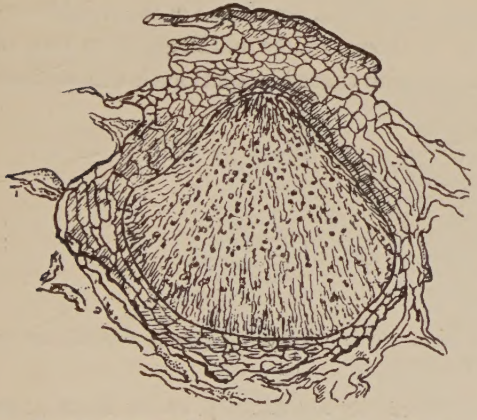

Fig. 5. Fungus isolated from fruit. Section of pycnidium on an inoculated fruit. ×500 diam.

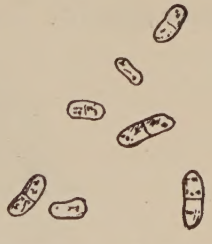

Fig. 6. Fungus isolated from fruit. Fresh spores from pycnidium on an inoculated stem. × 770 diam.

inserted and gradually spread throughout the tissues of the fruit. After 10-14 days numerous pycnidia developed on the surface. Fig. 5 shows a section of one of these pycnidia. The fruits remained

sound in control experiments. With ripe fruits, however, experiment showed that infection sometimes resulted when the mycelium was placed on uninjured parts.

Inoculations of wounded parts of stems of tomato plants were also made with the same mycelium. For this purpose a longitudinal slit was made in the stem about an inch above soil level and some mycelium was inserted, after which the wound was covered with tinfoil. All inoculations were performed as far as possible under

Fig. 7. Fungus isolated from stem. Fresh spores from pycnidium on an inoculated stem. ×770 diam.

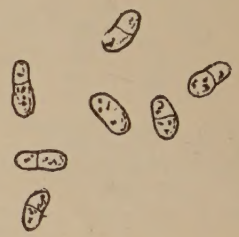

Fig. 8. Fungus isolated from stem. Fresh spores from pycnidium on stem infected in nature. ×770 diam.

sterile conditions. Other plants were kept as controls. The foliage of nearly every plant thus inoculated began to wilt about 7-8 days after the fungus was inserted. Fig. 12 shows one of the wilted plants and one of the control plants side by side. The affected plants subsequently died and in some of them pycnidia of the same character as those previously described, developed in the neighbourhood of the wound. Fig. 6 shows spores obtained from one of these pycnidia. In connection with these inoculations it was noticed

Fig. 9. Fungus isolated from stem. Section of young pycnidia on an inoculated fruit. ×125 diam.

that pycnidia were not formed so abundantly as in inoculation experiments made with the fungus originally derived from tomato stems. To make the story complete the fungus was plated out from these pycnidia and in cultures it proved to be identical with the fungus which had been used for inoculation. Upon examination of the tissues of plants which were successfully infected the

mycelium was found to have extended upwards and downwards both in the vessels and in the cortex. In the few plants which did not become infected after of inoculation, the mycelium had evidently failed to develop.

Inoculation Experiments with Fungus Derived from the Stem.

In almost every case when some of the mycelium grown on tomato-gelatine was inserted in a wound made in the stem of a healthy tomato plant, infection resulted and pycnidia of the same

Fig. 10. Fungus isolated from stem. Section of older pycnidium on an inoculated fruit. ×500 diam.

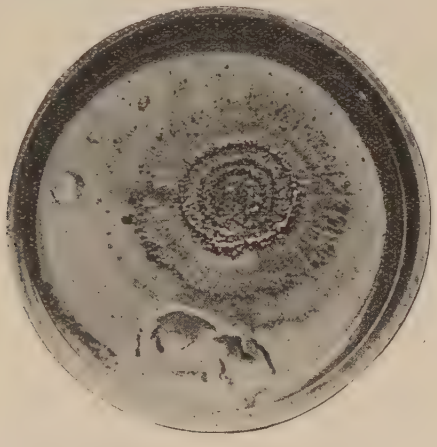

Fig. 11. Fungus isolated from stem. Photograph of culture on tomato-agar.

character as those previously described were formed in the tissues. Fig. 13 shows the wilting of a plant inoculated with this mycelium side by side with a control plant. The fungus plated out from these pycnidia agreed in cultural characters with the fungus which had been used for inoculation. Fig. 7 represents fresh spores obtained from these pycnidia. Fig. 8 shows fresh spores obtained from a plant infected in nature.

The same mycelium placed in wounded parts of the fruits caused rotting of the tissues and formation of pycnidia as previously described. The fungus plated out from these pycnidia was identical with that used for inoculation. Figs. 9 and 10 represent sections of these pycnidia in different stages of development.

The results of these inoculation experiments make it clear that the fungus causing a rot of the fruit is identical with that which induces canker on the stem.

Fig. 12. Photograph of plant inoculated with the mycelium of the fungus isolated from the fruit. A control plant is at the side.

Fig. 13. Photograph of plant inoculated with the mycelium of the fungus isolated from the stem. A control plant is at the side.

The Ascus Fructifications of the Fungus.

Grossenbacher[1] has shown in America that *Diplodina citrullina* (C. O. Sm.) Grossenbacher, or *Ascochyta citrullina*, C. O. Smith, as it has also been called, is the pycnidial stage of the Ascomycete,

[1] Grossenbacher, J. G., in N.Y. Agric. Expt. Sta., Geneva, N.Y., Technical Bull. No. 9 (1909).

Mycosphærella citrullina, Grossenbacher. This fungus causes a serious disease of melons in the United States, the pycnidial stage there being followed by the formation of perithecia which, however, do not develop until the tissues are dead or dying. We cannot find any record of the perithecial stage having been found in this country, and in the course of our own work perithecia were not seen, although plants and fruits killed by the fungus were kept in an exposed situation during the winter in order to try to induce their formation.

General Remarks.

As with so many other pathogenic fungi little is yet known of the manner in which infection by this fungus occurs in nature. The facilities which might enable such a matter to be solved have not been available to us. Such problems can be best studied in tomato houses and gardens developed on practical lines, although used primarily for experimental purposes. At present we do not know of any place in England where such work can be pursued in an adequate manner.

In one plot of out-door tomato plants in Cambridgeshire the disease occurred two years in succession, and in view of the apparent absence of a perithecial stage in this country and of the fact that the tomato plant is an annual, it is not clear how the disease is propagated from one year to another. It is possible that the mycelium hibernates in the dead tissues of affected plants, and it may be that portions of diseased plants left on the ground instead of being burnt are the means of reinfection if plants are set out on the same plot the following year. There is no evidence yet that the fungus is propagated in the seed. On out-door plants the disease does not appear to develop until almost fully grown. In the plots referred to above only 3 per cent. of the plants were affected and the distribution of diseased plants was sporadic, so that the disease is not likely to become a serious pest to growers of out-door tomatoes. In the case of tomatoes grown under glass, Massee[1] has already pointed out how severe an epidemic this fungus may cause on account of the conditions of growth being so favourable for its development. In consequence of its virulence under these conditions it is included in the list of pests scheduled by the Board of Agriculture, under the Destructive Insects and Pests Acts.

[1] Massee, G. *Kew Bulletin*, 1909.

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EDITED BY A. G. TANSLEY, M.A., F.L.S.,

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